UNPUBLISHED PRELIMINARY DATA

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Ryoichi Hayatsu

Enrico Fermi Institute for Nuclear Studies
University of Chicago, Chicago, Illinois

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Abstract. Purines, amino s-triazines, and substituted guanidines were isolated from the Orgueil meteorite, and were identified by chromatographic, spectroscopic, and other techniques. The presence of major amounts of s-triazine derivatives is of particular interest, since these compounds have no known biochemical significance.

In 1961, Calvin (1) and Briggs (2) independently reported spectroscopic evidence for purine and pyrimidine bases in water extracts of carbonaceous chondrites. Oró (3) criticized this work, suggesting that the observed spectral features were due to an impurity from the ion-exchange resin. Kaplan et al (4) found no conclusive evidence for the presence of these bases in meteorites from ultraviolet absorption data. In order to resolve this question, I attempted to isolate these compounds without use of ion-exchange resins.

Experimental Procedure and Results.

Sixteen grams of finely powdered Orgueil meteorite (5) which already had been extracted with water, ether, cyclohexane, chloroform, benzene, acetone, methanol, isopropanol, and n-butanol was extracted with 3N-HCl for 6 hours at 120°C in a sealed tube. After evaporation under reduced pressure, the dark brown residue, comprising 7.692 g, was acetylated by heating with acetic anhydride for 6 hours at 100°C, filtered, and the solvent removed in vacuo

below 30°C. The reddish brown residue (187.32 mg) was extracted with n-butanol. The alcohol extract was washed with cold aqueous sodium bicarbonate and cold water, dried, and the solvent removed. The residue was fractionated into four fractions with different organic solvents: fraction A (10.57 mg), chloroform soluble; fraction B (8.26 mg), hot chloroform soluble and cold chloroform insoluble; fraction C (86.03 mg), anhyd. methanol soluble and hot chloroform insoluble; fraction D (74.93 mg), soluble in 80% methanol and insoluble in anhyd. methanol.

The ultraviolet spectra of the fractions A, B, and C indicated the presence of amino derivatives of aromatic-type compounds which are pH sensitive:

Fraction A, $\lambda_{max}^{1N\ HCl}$ 238, 258 and 304 mμ; $\lambda_{max}^{alc.}$ 255-256 mμ; $\lambda_{max}^{1N\ NaOH}$ 253-256 (Sh = shoulder) mμ.

Fraction B, $\lambda_{max}^{1N\ HCl}$ 241, 315, and 362 mμ; $\lambda_{max}^{alc.}$ 252 (Sh) mμ; $\lambda_{max.}^{1N\ NaOH}$ 251-253 (Sh) mμ.

Fraction C, $\lambda_{max}^{1N\ HCl}$ 223, 250-252 (Sh) and 343-346 mμ; $\lambda_{max}^{alc.}$ 255 mμ; $\lambda_{max}^{1N\ NaOH}$ 252-256 (Sh) mμ.

The infrared spectra of fractions A, B, and C showed the presence of an amide carbonyl and a N-hetero aromatic ring (stretching vibrations 1600-1400 cm^{-1}):

Fraction A, ν_{max}^{KBr} 1685, 1610 and 1520 cm^{-1}

Fraction B, ν_{max}^{KBr} 1683, 1607 and 1512 cm^{-1}

Fraction C, ν_{max}^{KBr} 1685 (very weak), 1622 and 1535 cm^{-1}

Fraction A was purified on an alumina column (alumina, neutral, Brockman activity II 5 x 0.5 cm). Fraction A1, eluted with 70 ml of $CHCl_3$, consisted of 0.20 mg of colorless oil; fraction A2, eluted with 100 ml of $CHCl_3$, of 2.38 mg of pale yellow solid; and A3, eluted with 150 ml of $CHCl_3$-CH_3Oh 19:1, of 2.09 mg of yellow solid. Judging from the infrared and ultraviolet spectra, fraction A2 seemed to be a mixture, mainly of diacetylmelamine (6) and small amounts of other acetyl derivatives of N-heteroaromatic compounds:

Fraction A2, ν_{max}^{KBr} 3280-3175, 3160, 1732 (weak), 1686, 1615-1613 and 1511 cm^{-1}

$\lambda_{max}^{1N\ HCl}$ 234-236 and 255-256 (Sh) mμ

Diacetylmelamine, ν_{max}^{KBr} 3280-3200, 3165, 1685, 1615, 1572 and 1510 cm^{-1}

$\lambda_{max}^{1N\ HCl}$ 236 mμ

Fraction A2 was hydrolyzed with 5% HCl for 18 hours at 30°C. Solvent removal below 30°C gave 1.74 mg of the hydrochloride of the hydrolyzed product. Its yellow picrate was prepared, and was found to decompose between 297 - 310°C. Treatment of the picrate by conventional methods gave 0.63 mg of free base which was compared by thin layer chromatography with thirty-eight authentic purine, pyrimidine, and s-triazine compounds. (The chromatography was carried out on a silica-gel G plate activated at 105°C for 30 min., using a mixture of n-butanol-water-acetic acid 84:15:1 as the developer.) Seven spots were detected under

ultraviolet light and four of them were identified as melamine (Rf 0.26), ammeline (Rf 0.21), adenine (Rf 0.75) and guanine (Rf 0.62).

Fraction A3 was purified by the same procedure. The free base (0.402 mg), after purification through the picrate, was examined by thin layer chromatography. Three compounds identified were melamine, ammeline (7) and guanine, but five other spots remained unidentified. The approximate concentration of identified compounds is summarized in Table I.

Although fractions B and C have not yet been examined in detail, their spectra and behavior showed the presence of an N-heteroaromatic compound and an aliphatic amine. In particular, the infrared spectrum of fraction C indicated a very weak band in the region of amide carbonyl stretching absorption and this suggests incomplete acetylation under the conditions of this paper or decomposition of the acetylated product by alcohol or water. These compounds might be similar to uracil, hydroxy-purines with two or more OH-groups, ammelide, and some sort of guanidine derivative.

The infrared spectrum and elemental analysis indicated that inorganic compounds were present in fraction D. 70 mg of fraction D was fractionated further by dissolving in water and then extracting with n-butanol in a liquid/liquid extraction apparatus for a period of 36 hours. The alcohol extract was distilled under reduced pressure, leaving 4.75 mg of pale brown residue. After treatment of the residue with 3%-HCl for 10 hours at room tempera-

ture, the hydrochloric acid solution was evaporated in vacuo to dryness. To a solution of the hydrolyzed product in the minimum amount of water, 5 ml of alcohol was added, and the mixture was allowed to stand for 12 hours at 0°C. The amorphous compound which had separated was collected by filtration. After washing with cold alcohol and drying, it afforded 1.48 mg of the hydrochloride (fraction D-1). The infrared spectra of the hydrochloride and its free base showed that typical guanidine compounds were present. In particular, the spectrum of the hydrochloride showed features at 3422-3295, 1651, 1612 and 1535 (Sh) cm^{-1}, and thus strongly resembled the spectra of N, N- and N'-disubstituted guanidine hydrochlorides.

In connection with the characterization of fraction D-1, the infrared spectra of twenty-six hydrochlorides of primary and secondary amines and guanidine were measured. Further, the published infrared spectral data of over one hundred amine hydrochlorides were compared with the spectrum of the meteoritic sample. The primary amino group gives rise to NH-stretching bands in the region of 3500-3300 cm^{-1} and the NH-stretching bands of its ammonium ion occur below around 3250 cm^{-1} (8,9), whereas the NH-stretching bands of the guanidinium ion appear in the free amino region rather than in the ammonium region (9,10). This suggests that the positive charge is localized mainly on the central carbon atom rather then being distributed over the entire guanidinum group. Generally, mono- (NR-) and disubstituted (NR-, N'R-) guanidine hydrochlorides give two bands between 1690

and 1565 cm^{-1} due to the NH- deformation vibrations, while unsubstituted guanidine hydrochlorides (guanidine itself) and trisubstituted ones (NR-, N'R'-, N"R-) show only one band in the deformation region. The positive Sakaguchi reaction (11) indicated that mono-substituted guanidine groups are also present in this fraction. No characteristic ultraviolet absorption was detected in this fraction except for a very weak shoulder in the region of 275-285 mμ. From these data, it was deduced that mono- and disubstituted guanidines were present in fraction D-1.

Thin layer chromatography of fraction D-1 gave six spots. Although these spots have not yet been identified, neither guanidine itself nor its simpler alkyl-substituted derivatives were detected on the chromatogram. Presumably, fraction D-1 consists of guanidine derivatives of iminazolidine, iminazaline and iminazole. We know that the acid hydrolysis of adenine (12) or guanine (13) leads to the formation of iminazol derivatives under mild conditions. Conceivably the guanidine derivatives found may have formed as degradation products of nucleic acids or other compounds, but this possibility was not yet been investigated.

Table I. Identified compounds in Orgueil meteorite, μg/g dry weight

	Fraction A2	Fraction A3
melamine	15 - 25	10 - 20
ammeline	8 - 18	10 - 20
adenine	10 - 20	
guanine	6 - 16	4 - 14
Total	39 - 79	24 - 54
Total	63-133	

Discussion.

What bearing do these results have on the purported evidence for life in meteorites? First, the presence of purines has now been unambiguously confirmed. Second, there is the problem of accounting for the origin of the melamine and ammeline. Neither these compounds, nor others based on the s-triazine ring system have any known biological significance. Either they were produced by an extraterrestrial life form whose biochemical pathways differ from those of terrestrial life, or they were made by abiotic processes. The former alternative cannot be excluded a priori, but it runs counter to all recent arguments for a biological origin of the meteorite organic compounds. These arguments have cited the similarities between meteoritic organic matter and terrestrial biogenic materials as evidence for extraterrestrial life with a biochemistry closely duplicating the terrestrial one. It is somewhat puzzling that a biochemistry differing, ex hypothesi, from our own in one major respect, should resemble it so closely in all others.

On the other hand, there seems to be no problem in accounting for the s-triazine derivatives by abiotic processes (14, 15, 16). Such compounds can, in principle, be synthesized from hydrogen cyanide and ammonia or from a simple organic molecule under the conditions thought to have prevailed in the early solar system. Melamine can be prepared by chemical synthesis from (a) hydrogen cyanide and ammonia, (b) guanidine carbonate and dicyandiamide, (c) dicyandiamide itself, (d) guanidine or biguanide,

(e) urea, (f) carbon monoxide and ammonia, and (g) many others. Although the mechanism of melamine formation from the above starting materials has not yet been worked out in detail, the reaction might proceed through an active intermediate such as cyanamide. Mechanisms for the formation of adenine from hydrogen cyanide and ammonia under primitive earth conditions have been suggested by Calvin (1) and Oró (17), and several successful syntheses from hydrogen cyanide, ammonia, and other simple compounds have been reported (17, 18, 19). Guanine was also formed (20) by thermal polymerization of amino acids. Studies on the synthesis of melamine and other organic compounds are being conducted in our laboratory, and will be reported in due course (21).

References and Notes

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6. Acetylation of melamine gives only the diacetyl derivative under the conditions used in this work.
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